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A randomized crossover feeding trial with a high monounsaturated fat diet (HIMUFA) based on macadamia nuts and oil and a typical American diet high in saturated fat (HISAT) was conducted on 27 healthy men and women aged 20 to 52. Fatty acid profiles for the 2-diets were HIMUFA, 28% Kcal fat (22% MUFA, 9% SFA); HISAT, 38% Kcal fat (11% MUFA 20% SFA). The percentage of calories derived from protein and carbohydrates were 15% and 47% respectively, and cholesterol levels were constant (300 mg/day) for both diets. Each diet was consumed for 8 weeks with a 2-month washout period. The preliminary data showed that the isoenergetic substitution with 11% Kcal MUFA for SFA significantly reduced plasma total cholesterol (mean±se,-23.6±3.2 mg/dl, $p<0.0001$), LDL-C (-12.4±2.9mg/dl, $p<0.0002$) as well as HDL (-9.1±1.6mg/dl, $p<0.0001$). Changes in triglyceride levels were not significant. These results support the beneficial effect of long-term consumption of diets high in MUFA.

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EFFECTS OF DIETS HIGH IN PALMITOLEIC ACID ON SERUM LIPID LEVELS AND METABOLISM, Phase 2

I. BACKGROUND

Monounsaturated fatty acids have been demonstrated to have the potential of providing high energy density without the consequences of atherosclerosis and increased cancer risk associated with other high fat diets. Palmitoleic acid appears to be especially promising in this regard, and palatable food products high in this fatty acid are produced in the United States.

Previous animal and human studies have indicated that increased proportions of monounsaturated fats in the diet may have beneficial effects on serum total cholesterol and HDL/LDL ratios. Yamori, et. al. reported that a diet containing 1% palmitoleic acid reduced the stroke rate and increased survival in stroke-prone spontaneously hypertensive rats despite increased salt intake to a greater extent than the unsaturated fatty acid linoleic acid.[1] The Seven Countries Study[2] reported low rates of coronary heart disease in countries where fat intake was high, but consisted mainly of monounsaturated fatty acids. Grundy[3] concluded that substitution of monounsaturated fats for saturated fats in the diet may make it unnecessary to resort to diets very low in fat to achieve optimal reduction in plasma cholesterol levels. Diets rich in monounsaturated fats can reduce total cholesterol and LDL cholesterol levels without the associated reduction of HDL cholesterol levels seen in diets very low in fat or high in polyunsaturated fat.[4,5,6,7] Furthermore, diets high in polyunsaturated fatty acids have been found to promote tumor development after pretreatment with chemical carcinogens[8] and to suppress the immune system[9,10] in laboratory animals. Unlike diets high in either saturated or polyunsaturated fats which have been associated with increased cancer risk at multiple sites, monounsaturated fat intake did not correlate with increased cancer risk at any site.[11]

Macadamia nuts are among the natural foods known to contain a high proportion of the monounsaturated fat, palmitoleic acid. A pilot study was conducted in 1992 to

determine how regular consumption of this food would affect human volunteers. Seventy-four healthy, free-living subjects, 30 to 75 years of age and having a serum cholesterol level >200 mg/dl were randomly assigned to low dose (1.6 oz/day); high dose (3.2 oz/day); or a normal diet control group for 4 weeks. After 4 weeks there were no significant changes in body weight, serum lipids, fasting glucose, laboratory measures of kidney or liver function, or blood pressure in the 3 groups. One person assigned to the high dose group reported severe diarrhea and refused to continue. This subject was included in the high dose group for statistical analysis even though he ate no nuts. For the remainder of the subjects, side effects were usually described as minimal and were elicited only on detailed questioning about gastrointestinal symptoms.

Based on this pilot study and on the results and advice of an international workshop with a group of recognized experts in the field of nutrition research organized by the researchers, a double blind randomized cross-over study with three diets was undertaken (phase one). Diet A was a "standard American diet" with 37% of calories from fat (16% saturated, 7% polyunsaturated, and 14% monounsaturated). Diet B was the American Heart Association "Prudent diet" with 30% of calories from fat (9% saturated, 7% polyunsaturated, and 14% monounsaturated). Diet C was the macadamia diet with 37% of calories from fat (9% saturated, 7% polyunsaturated, and 21% monounsaturated). Each diet was given in random sequence for four weeks. A six day run-in period preceeded the first experimental diet period. Twenty-three men and nineteen women began the study. Sixteen men and eighteen women completed the run-in period and began the experimental diets. Fifteen men and fifteen women completed the study. Those subjects who left the study left for personal reasons or for inability to adhere to the meal plan. No subject left the study for side effects. Compared to the "standard American diet", both other diets resulted in very similar and significant reductions in total and LDL cholesterol. Triglycerides increased significantly in the low fat diet, but decreased significantly in the high monounsaturated fat diet.[7]

TABLE 1. Average lipid values (S.D.) for the three dietary regimens of the Diamond Head Nutrition Research Study.

	"Typical American"	Moderate Fat Diet	Macadamia Diet
Total cholesterol	201.2 (30.4)	193.1 (34.5)[a]	191.3 (32.6)[a]
LDL cholesterol	130.4 (25.7)	124.3 (30.4)[a]	124.5 (29.5)[a]
HDL cholesterol	55.3 (7.6)	52.0 (8.2)[a]	52.8 (8.2)[a]
Triglycerides	77.5 (32.7)	83.6 (32.6)[a]	70.4 (26.0)[a]

a = $p < 0.05$ compared to "Typical American" diet in a linear models analysis.

II. HYPOTHESIS

Substitution of the monounsaturated fatty acid, palmitoleic acid, for saturated dietary fat will result in a healthier blood lipid profile regardless of total fat intake if total calorie intake and expenditure remain balanced. This effect can be maintained over an extended period of time without appreciable adverse effects.

III. TECHNICAL OBJECTIVES (phase 2).

A group of 34 men and women age 20 to 51 was recruited to participate in a randomized study of 2 diets which was fed to each individual for 8 weeks in random sequence in a cross over design. A 5 day run in period preceded randomization. Of the 34 men and women who initially enrolled in the project 27 completed the study. Thus each individual who successfully completed the study ate only study prepared meals for approximately 17 weeks.

It was expected that results would show a significant improvement in the blood lipid profile and in hemostatic factors during the diet high in palmitoleic acid without significant changes in other metabolic parameters such as glucose tolerance.

IV. MILITARY SIGNIFICANCE

The study investigates further the use of energy dense dietary products prepared from natural products available in the United States and high in the monounsaturated fatty acid palmitoleic acid, for use by the men and women of our armed forces. The development of healthier, yet acceptable energy dense dietary products for use in field and combat situations as Meals Ready to Eat (MRE) would contribute to simplified logistics while providing greater potential long term fitness. Such products might be especially applicable for Rations Lightweight (RLW) and for restricted Rations MRE's.

V. METHODS (Recruitment, compliance and operations)

Informed Consent

Use of human subjects was reviewed and approved by the human research committees of the University of Hawaii and other participating institutions. Informed consent was obtained from each subject after a brief screening history for exclusion criteria and before any blood tests are done or any diet given. Informed consent by proxy was not be accepted.

Inclusion/Exclusion Criteria

All potential subjects were screened for health exclusions including having relatively low blood cholesterol, which may be less responsive to dietary intervention. Subjects were between 20 and 51 years of age, and had a documented serum total cholesterol between 180 and 240 mg/dl. Documentation was provided be the use of a screening cholesterol measurement. A history of allergy to tree grown nuts, diabetes mellitus or pancreatic insufficiency, fasting hypertriglyceridemia over 400 mg/dl at baseline, current pharmacologic treatment for hyperlipidemia, or an unstable medical condition of any kind were grounds to exclude the volunteer from further participation in the program. Subjects on medication for chronic medical conditions (i.e. chronic replacement therapy for hypothyroidism, use of certain oral contraceptives, stable and

controlled mild to moderate hypertension) were allowed to continue if the medication was long-term and was unlikely to change in dosage or character during the course of the project. Pregnancy and breast feeding were exclusionary criteria for this study.

Baseline Evaluation

The baseline evaluation consisted of a health, dietary and family history, a brief physical examination; laboratory tests including fasting blood sugar, serum lipids, kidney, liver and thyroid function; and a serum test for pregnancy for female subjects. Reports of all findings were given to each volunteer.

Recruitment and compliance

Recruitment of subjects began August 25, 1997 and continued through September 22, 1997. Recruitment consisted of posters and flyers being placed on bulletin boards throughout the University as well as advertisements being placed in the Universities student newspaper. Volunteers were directed to contact a message telephone number if they were interested in receiving more information about the study. Approximately 400 inquires were received by project staff during the course of the recruitment period.

Each message inquiry was followed up by research staff who contacted participants and provided them with a description of the project as well as the required commitments needed from volunteers in order to become study subjects. If the participant expressed continued interest in the study the telephone interviewer conducted a telephone survey questionaire and scheduled an appointment to meet with project staff at the East West Center.

The first appointment at the East West Center was to review the study protocol and receive consent to perform a screening blood draw to determine cholesterol levels. Subjects with initial screening cholesterol levels significantly under 180 were immediately eliminated for consideration. Selected subjects with cholesterol levels at or near 180 were scheduled to meet with the study nutritionist to discuss further the requirements of the study and commitment necessary for participation.

Operations Overview

The success of a nutrition intervention program is largely due to the amount of participant contact time and the skill of the nutritional interventionist. Jenny Tung, MS, the nutrition epidemiologist was on site to provide the individual instruction and counselling necessary to deliver that intervention over the period of the trial. Social learning theory and behavioral modification approaches have enhanced the understanding of behavioral changes such as those required in successful dietary programs. The integration of these approaches with more traditional dietary counselling has resulted in techniques which have been successful in research settings.

Dietary Intervention

The dietary intervention was a controlled, double blinded crossover design feeding study of 2 dietary options lasting 8 weeks each. Originally 34 participants were ramdomization into the 2 diet sequence group by block according to their sex. Participants received a $600 stipend for successful participation. Partial compensation was provided on a proportional basis for those participants withdrawing early.

A run-in period of 5 days, in which an "average American" diet was eaten, screened participants for compliance and willingness to accept the restrictions imposed by the dietary regimens. One participant was removed from the study at run-in due to concerns about compliance. The experimental diets were: 1) a high monounsaturated fat diet with 38% of calories as fat (9% saturated, 7% polyunsaturated and 22% mono-unsaturated fats) based on naturally occurring food products with a high content of palmitoleic acid, and 2) a "typical American" 38% fat diet (20% saturated, 7% polyunsaturated, and 11% monounsaturated fats). The percentage of calories derived from protein, and carbohydrate was held constant in each diet. Cholesterol levels were held constant for both diets.

The composition of each diet for four levels of energy intake, ranging from 1500 calories to 3500 calories, was planned by computer using the Food Processor II (ESHA

Research, Salem, Oregon) and the Nutrition Data System (University of Minnesota) software programs for natural and common foods. The foods in the high monounsaturated fatty acid diet were similar to those in the usual American diet with reduced portions of fatty foods and visible fats, some or all of which were replaced by products high in palmitoleic acid.

Additional calories were provided in the form of "unit foods". These were in the form of 100 kcal cookies, 150 kcal muffins and 200 kcal scones. Unit foods were developed to match the nutrient profile for each diet. Participants were allowed to eat up to three of these "unit" foods per day in addition to their diet regimen as long as they maintained their bodyweight.

A limited amount of non-caloric caffeinated beverages were allowed (up to five cups of coffee/day). All other beverage were required to be non-caloric decaffeinated. Up to five alcoholic drinks (wine, beer, or whiskey) were allowed per week.

Consumption of all foods and beverages were recorded in the daily dairy. It included the items and the amounts of all the foods and beverages consumed outside of those provided by the study.

Daily energy intake needed to maintain weight was estimated for each subject according to Harris-Benedict equations by an activity factor and compared to calorie intake on three-day food records completed immediately prior to the run-in period. The calculations were compared with body weight measurements and caloric levels were altered when necessary to maintain each subject's weight.

Subjects in both diets received at least 95% of all food consumed from the study kitchen. On weekdays, subjects ate breakfast in the study dining room which was located at the East West Center on the University of Hawaii campus. Lunches were packed in containers and given to subjects as they left breakfast. If requested these lunches were

stored at the facility kitchen for pickup by participants during lunch time. Dinners were eaten at the study dining room.

On weekends subjects ate brunch in facility dining room. Saturday dinner was declared a "free" meal. Subjects could eat a dinner prepared by kitchen staff and taken home, or could eat a non-study prepared meal at their home or restaurant. The purpose of this meal was to provide a small break from the rigors of compliance so as to maintain subject morale. This non-study prepared meal had to follow guidelines provided by the study nutritionist and was recorded in detail on the daily diary. During the course of the study over 50% of the subjects elected to take home a study prepared dinner on Saturdays. On Sunday's, participants were provided a study prepared dinner to be taken with them after they had completed their Sunday brunch.

Compliance

Subjects maintained their physical activity and other lifestyle habits constant. These factors were monitored through a diary provided by the study to record illness, medication use, and deviations from their usual physical activity patterns or from the study diet. Each subject received specific training on how to keep the diary. Diaries were reviewed daily by the study nutritionist. A dedicated telephone line was established for this study and the subjects asked to call to report any problems to the study nutritionist during normal business hours. The study nutritionist was available at all times by pager. After hour contact with the study nutritionist was minimal. There were no significant problems, medical concerns, or serious adverse events.

Measurements

Blood pressure, weight measurement, and blood drawing were conducted in a small clinic located on the second floor of the East West Center. This room was set up for use by Manoa Valley Nutrition Research staff only. All used supplies and waste materials were appropriately packaged, labelled and returned to the University of Hawaii for disposal. Specimens for laboratory analysis were transported to study repository located at Kuakini Medical Center and stored in a repository freezer's at -70 degrees.

Weight was measured in street clothes, without heavy clothing or shoes, at the feeding site once during the run-in period and once a week during the study period. Energy intake was adjusted to maintain a stable body weight. During the entire study period there was no average weight gain.

Blood pressure was measured each time weight was measured. Two readings were taken in the sitting position after five minutes of rest with a standard sphygmomanometer by certified study personnel trained and standardized by protocols and methods.

On the fifth week of each feeding session, before breakfast and after an overnight fast of not less than 12 hours, each subject had their blood drawn. On two successive days of the eighth week of each feeding session, before breakfast and after an overnight fast of not less than twelve hours, each subject had their blood drawn Bloods were drawn and processed using trained phlebotomist and lab technicians in the portable clinic and lab facility located on the second floor of the East West Center**0**All blood draws were uneventful.

Blood Analysis

1. Lipid Panel

Analysis of blood specimens was conducted by the University of Hawaii, Division of Medical Technology in the Summer of 1998. This lab participates in the lipid standardization protocal administered by the Centers for Disease Control (CDC). The analysis included plasma TG, TC, HDL, LDL and glucose. Specimens were blinded and

included duplicates as well as Standard Reference Material provided by the National Institute of Standards and Technology. These additional specimens served as quality control for laboratory procedures. Specimens were frozen to minus 70 degrees as part of the initial processing. These specimens were packed in dry ice and hand delivered to the University of Hawaii, Division of Medical Technology. They arrived at the University in a frozen condition and then were stored on-site at minus 70 degrees until the analysis was conducted.

TC, TG, HDL, and glucose were quantified with CDC standardized enzymic assay in a Beckman Synchron CX4 automated analyzer. LDL was derived by Friedewald estimation since the TG in all speciman were less then 400mg/dl. The Friedewald estimation equation is:

LDL cholesterol = Total cholesterol – HDL cholesterol – Triglycrides/5

2. Lipoprotein particle size

In January of 1999 additional specimens were sent to Lipomed, Inc. in Raleigh, North Carolina for further analysis using nuclear magnetic resonance (NMR) spectroscopy. This analysis included a lipid panel (VLDL, TG, TC, HDL, LDL), particle size (HDL, LDL), and 16 subclasses of HDL, LDL, IDL and VLDL. Frozen samples were packed in dry ice and sent by Federal Express to Raleigh, North Carolina. These specimens arrived at Lipomed, Inc. in a frozen condition and were stored at minus 70 degress until analysis was conducted. This analysis was completed in February of 1999. Data is subsequently being analyzed.

Food Analysis

Three samples of each study diet were randomnly collected during the study period. These samples were stored at the Kuakini Medical Center repository in freezers at minus 70 degrees. Woodson-Tenant Laboratories, Inc. in Dayton, Ohio was selected to conduct laboratory analysis of nutrient content of these food samples. Analysis included

moisture, fat, protein, fiber, ash, calories (bomb calorimeter), cholesterol, and fatty acid composition.

Frozen samples were packed in dry ice and sent by Federal Express to Dayton, Ohio. The samples arrived at Woodson-Tenent Laboratories, Inc. in a frozen condition and were stored at minus 70 degress until analysis was conducted. This analysis was completed in December of 1998.

The methods of the analysis for each components are as followed:

Fat	AOAC 954.04
Fatty acid	AOCS Ce-1e-91
Protein	AOAC 990.3
Fiber, crude	AOAC 962.09
Carbohydrate	AOAC 962.09
Ash	AOAC 923.03
Moisture, vacuum	AOAC 943.01
Cholesterol	AOAC 976.26
Bomb calories	Parr Calorimeter

Statistical Analysis

Differences in lipid panel as well as glucose were compared by using a paired t-test. The means of these values form two blood samples at the end point were compared for the analysis. Data was analyzed using the SAS program 6.12. P values less than 0.05 was considered to indicate statistical significance. All significance levels were bases upon two-tailed test.

VI. STUDY RESULTS

Study Participants

Thirty four partcipants (17 men, 17 women) were selected to enter the run-in portion of the study. Interviews were conducted between the subjects and the study nutritionist during run-in. Ultimately one participant (a male) was excluded due to

concerns regarding compliance with the study protocol. From this run-in pool thirty-three participants entered the feeding sessions. In week four one participant (a male) from the study due to unforeseen difficulties in traveling to the University each day. At the end of the first feeding seesion and during the 60 day washout period five participants (3 women and 2 men) dropped from the study citing personal difficulties in maintaining compliance with study protocols. Twenty seven participants completed the studies second feeding session.

Overall there was no significant weight change during the study periods. The mean and standard deviation of body weight changes were 0.22 ± 1.55 lb. in men, and was 0.4 ± 2.26 lb. for women. The changes of blood pressure in SBP and DBP were not statistically significant, althought both SBP (-0.96 ± 1.21 Hg) and DBP (-1.12 ± 0.86 Hg) tended to fall in the high monounsaturated diet as compared to the typical american diet.

There were 13 men and 14 women who completed the feeding sessions. Their ages ranged from 20 to 51 years with the mean age being 32.5 years for men and 28.4 years for women (Table 2). The mean body mass index (BMI) for men and women were 26.4 and 22.5, respectively. Overall, the mean baseline cholesterol were nomal to high. The mean and the standard deviation of baseline cholesterol were 208 ± 14mg/dl for men. For women, the mean baseline cholesterol were 193.8 ± 21.2 mg/dl. Approximately 30 percent of the completed partipants were Caucasion, 56 percentage were Asian-Pacific Islander, and 15 percent were mixed with Asian and Caucasion.(Table 3). Only two people who participated in the study were current drinkers or smokers (Table 4). Three out of the 14 women (21%) took birth control pills during the study period.

Food Analysis

A comparison between the analyzed mean of the daily macronutrient compostion, as well as cholesterol and fiber, of the 12-days cycle menu and the calculated nutrition profile, using the Genesis and NDS software, is shown in Table 5. The major components of our research diet, the fatty acid profile, was determined by analysis to be very close to

the profiles calculated using the nutrition software packages. Other than a significance differences in calories derived from saturated fatty acid and monounsaturated fatty acid as designed in the study, the two study diets had similar nutritent contents except for fiber. In addition, the individual fatty acid content analysis confirmed the expected pattern of the major fatty acids distribution for each diet as shown in Table 6.

Blood Analysis

The diet effects on lipid levels for the overall group and for groups divided by sex are shown in Table 7 and Table 8. The reductions in plasma total cholesterol (mean ± se, -23.6 ± 3.2mg/dl), LDL (-12.4 ± 2.9 mg/dl), HDL (-9.1 ± 1.6 mg/dl) in the high monounsaturated diet as compared to the typical American diet were all statistically siginificant for the overall group ($p < 0.0002$). These reductions in TC, LDL, and HDL were equal to 10.9%, 10.1%, and 12.3% of the values for the typical American diet respectively. Both plasma triglyceride and glucose did not change significantly although they tended to decrease in the high monounsaturated fatty acid diet as compared to the control diet. The patterns of the diet responses were similar in both sexes although the differences were not consistently significant. The smaller sample sizes and greater variablility may have contributed to the discrepency in the results.

References/Bibliography

1 Yamori Y, Nara Y, Tsubouchi T, Sogawa Y, Ikeda K, Horie R: Dietary prevention of stroke and its mechanisms in Stroke-Prone Spontaneously Hypertensive Rats - Preventive effect of dietary fibre and palmitoleic acid. 1986, J Hypertension 4(suppl 3):S449.

2 Keys A, ed: Coronary heart disease in seven countries. 1970, Circulation 41(suppl 1):I-1.

3 Grundy S: Monounsaturated fatty acids, plasma cholesterol, and coronary heart disease. 1987, Am J Clin Nutr 45:1168.

4 Grundy SM: Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. 1986, N Engl J Med 324:745.

5 Mensink RP, Katan MB: Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. 1987, Lancet 1:122.

6 Mattson FH, Grundy SM: Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. 1985, J Lipid Res 26:194.

7 Curb JD, Wergowske GL, Dobbs J, Abbott RD, Huang B: Comparison of lipid levels in humans on a macadamia nut based high monounsaturated fat diet to their levels on a moderate fat diet and a high fat "typical American" diet. Accepted, American Heart Association Scientific Conference on the Efficacy of Hypocholesterolemic Dietary Interventions, San Antonio, TX, May 4, 1995.

8 Carroll KK, Khor HT: Effects of level and type of dietary fat on incidence of mammary tumors induced in female Sprague-Dawley rats be 7,12-dimethyl benz(a)anthracene. 1971, Lipids, 6:415.

9 Meade CJ, Merten J: The mechanism of immunoinhibition by arachadonic acid and linoleic acid. Effects on the lymphoid and RE systems. 1976, Int Arch Allergy Appl Immunol 51:512.

10 De Vries CE, van Noorden CJ: Effects of dietary fatty acid composition on tumor growth and metastasis. 1992, Anticancer Res 12:1513.

11 Hursting SD, Thornquist M, Henderson MM: Types of dietary fat and the incidence of cancer at five sites. 1990, Prev Med 19:242.

Tables 2 - 8

TABLE 2 : MVNRS Baseline Information-Study Population - Demographics

	Males (n=13)	Females (n=14)
Age (years)	32.5±9.9	28.4±6.7
BMI	26.4±3.2	22.5±3.2
Cholesterol	208.8±14.0	193.8±21.2
Systolic BP	124.6±8.1	117.3±8.0
Diastolic BP	82.0±5.2	78.2±8.3
Education:		
Undergraduate student	7	8
Bachelor's Degree	0	2
Graduate Student	6	4
Housing:		
Off Campus	9	8
On Campus	3	6
Mixed	1	0

TABLE 3:MVNRS Baseline Information Study Population – Ethnicity

	Males (n=13)	Females (n=14)	Percentage (n=27)
Caucasian	3	5	29.6
Chinese	1	4	18.5
Japanese	4	1	18.5
Korean	0	2	7.4
Asian Indian	1	0	3.7
Pacific Islander	1	0	3.7
Mixed	2	2	14.8
Filipino	1	0	3.7

TABLE 4: MVNRS Baseline Information Study Population - Social Habits

	Males (n=13)	Females (n=14)
Drinking:		
Never	5	6
Current	7	6
Not current	1	1
Unknown	0	1
Smoking:		
Never	12	11
Current	1	1
Not current	0	2
Using Birth Control Pill:		
No	13	11
Yes	0	3

TABLE 5 : Mean daily nutrient composition if the two study diets*

Variable	Control Diet		High MUFA Diet	
	Calculated	Analyzed	Calculated	Analyzed
Energy (Kcal/day)	2000 ± 0.4	1921 ± 78	2000 ± 0.4	1941 ± 65
Fat (% of Kcal)	38 ± 0	36.7 ± 2.0	38 ± 0.0	38 ± 2.2
Saturated fatty Acid	20.4 ± 2.9	19.5 ± 2.6	7 .8 ± 0.5	8.5 ± 0.5
Monounsaturated fatty acid	8.5 ± 0.5	9.3 ± 2.5	22.4 ± 0.5	24.2 ± 1.3
Polyunsaturated fatty acid	6 ± 0.6	6.5 ± 0.9	5.3 ± 0.7	5.5 ± 0.4
Protein (% of Kcal)	15 ± 0.0	14.5 ± 1.1	15.0 ± 0.0	15.1 ± 1.5
Carbohydrates (% of Kcal)	46.9 ± 0.3	48.8 ± 2.6	46.9 ± 0.3	46.9 ±3.1
Cholesterol (mg/day)	300 ± 0.3	268 ± 27.8	300 ± 0.6	275 ± 24.5
Fiber (g/day)	15.4 ± 1.6	7.7 ± 2.6	16.3 ± 1.4	11.7 ± 5.3

* All values denote mean ± SD of the 12-day cycle menu
Control diet denotes "Typical American Diet"
High MUFA diet denotes "High Monounsaturated Fatty Acid Diet"

TABLE 6: Proportions of the major fatty acids in the two study diets*

	Fatty Acid	Control Diet %	High MUFA Diet %
SFA	C12:0	0.65 ± 0.21	0.03 ± 0.03
	C14:0	0.41 ± 0.08	0.07 ± 0.02
	C16:0	0.92 ± 0.13	0.66 ± 0.07
	C18:0	0.37 ± 0.06	0.25 ± 0.03
MUFA	C16:1	0.07 ± 0.08	0.65 ± 0.05
	C18:1	1.15 ± 0.24	2.56 ± 0.19
PUFA	C18:2	0.79 ± 0.08	0.69 ± 0.08
	C18:3	0.06 ± 0.03	0.04 ± 0.02

* All values denote Mean ± SD of the weight percentage of the 12-day cycle menu.

TABLE 7: Diet effects on plasma lipids and glucose*

	Control Diet	High MUFA Diet	Diet Effect
Total Cholesterol	215.2 ± 22.3	191.6 ± 19.3	-23.6 ± 16.5[a]
LDL-C	122.5 ± 28.9	110.7 ± 22.0	-12.4 ± 14.8[b]
HDL-C	73.6 ± 21.9	64.5 ± 18.3	- 9.1 ± 8.4[a]
Triglycerides	86.6 ± 44.8	85.0 ± 51.2	- 1.6 ± 15.8
Glucose	94.6 ± 8.6	92.7 ± 9.6	-1.6 ± 4.7

All value denote Mean ± SD mg/dl.
The diet effect denotes High MUFA diet vs. Control diet
a : $P < 0.0001$
b : $P < 0.0002$

TABLE 8: Diet effects on Plasma Lipids and glucose by sex*

	Control diet	MUFA Diet	Diet Effect
Total Cholesterol			
Men	213.4 ± 18.5	191.2 ± 19.6	-22.2 ± 11.4[a]
Women	216.9 ± 25.9	191.9 ± 19.8	-25.0 ± 20.6[b]
LDL-C			
Men	134.5 ± 18.8	119.4 ± 20.1	-15.2 ± 5.5[a]
Women	111.3 ± 32.6	101.5 ± 20.8	- 9.9 ± 19.9
HDL-C			
Men	57.7 ± 15.1	49.7 ± 11.4	- 8.0 ± 8.9[c]
Women	88.4 ± 16.2	78.3 ± 11.2	-10.0 ± 8.1[b]
Triglycerides			
Men	105.9 ± 52.7	111.1 ± 59.0	5.2 ± 18.6
Women	68.8 ± 27.2	60.8 ± 26.2	-8.0 ± 9.5[b]
Glucose			
Men	99.5 ± 7.2	98.3 ± 9.3	-1.2 ± 4.5
Women	89.3 ± 6.9	87.5 ± 6.7	-1.9 ± 5.1

All values denote Mean ± SD mg/dl
Diet effect denotes the high MUFA diet vs Control diet
a : $P < 0.05$
b : $P < 0.005$
c : $P < 0.0001$

Presentation of Study Results

In the past year there have been five presentations of study results locally and nationally. These presentations were:

1. "High Monounsaturated Fat Macadamia Nut Diet Effects on Serum and Lipoproteins" - JD Curb, MD, G. Wergowske, MD, RD Abbott, PhD, JC Dobbs, PhD, KH Tung, MS, M Austin, PhD, S. Marcovina, PhD, C. Waslein, PhD

Presented at the Experimental Biology, 98 meetings in San Francisco, California by Dr. J. David Curb.

2. "A High Monounsaturated Fat (MUFA) – Macadamia Nut Diet (MAC) and Serum Lipids and Lipoproteins" - C. Waslein, PhD, JD Curb, MD, G. Wergowske, MD, RD Abbott, PhD, MA Austin, PhD, S. Marcovina, PhD, KH Tung, MS

Presented at the University of Hawaii, School of Public Health 1st Annual Poster Session by Ko-hui Tung.

3. "High Monounsaturated Fat Macadamia Nut Diet Effects on Serum and Lipoproteins" - JD Curb, MD, G. Wergowske, MD, RD Abbott, PhD, JC Dobbs, PhD, KH Tung, MS, M Austin, PhD, S. Marcovina, PhD, C. Waslein, PhD

Presented as a poster at the Hawaii Dietetic Association meetings in Honolulu, Hawaii by Ko-Hui Tung.

4. "Clinical Trial to Determine the Ability of a Diet High in Monounsaturated Fatty Acids to Reduce Risk Factors for Coronary Heart Disease" - KH Tung, MS

Presented at the 1998-99 University of Hawaii, School of Public Health Colloquium Series by Ko-Hui Tung.

5. "Long-Term Effects of Diets High in Monounsaturated Fatty Acid on Plasma Lipid Levels in Healthy Men and Women. The Macadamia Nut Study, Phase II."- KH Tung, MS, C. Waslein, PhD, PJ Elmer, PhD, RD Abbott, PhD, G. Wergowske, MD, JD Curb, MD

To be presented at the Experimental Biology '99 meetings in Washington, D.C. by Ko-Hui Tung.

List of all personnel who received pay from the contract

Name (Last name,	first name)	Job Title
Baldino,	Ruth	Recruiter
Bonilla,	Benecito	Cook helper
Campos,	Violet	Cook helper
Dibas,	Chester	Cook
Domdom,	Dinna	Project Coordinator
Duldulao,	Lolito	Cook
Flint,	Mary	Cook
Franklin,	Buddy	Cook helper
Hein,	Evelyn	Project Coordinator
Hsia,	Liang-ho	Cook helper
Jardine,	Sunny	Dishwasher
Kane,	Rod	Cook
Monje,	Sanny	Cook helper
Nakagawara,	Linda	Cook helper
Nakamura,	Troy	Cook
Nakata,	James	Cook
Nicholson,	Diane	Phlebotomist
Pacis,	Ceasar	Cook
Poon,	Sau-ling	Cook helper
Rabanal,	Ofelia	Lab Tech.
Resgonia,	Daryn	Cook
Richards,	Allison	Lab Tech.
Sagon,	Dionicio	Baker
Shigeta,	Sheryl	Lab Tech.
Takamoto,	Derek	Head Cook
Thomas,	Dale	Cook
Tung,	Ko-hui	Nutritionist/Epidemiologist